Mini review

Structure-antioxidant and anti-tumor activity of *Teucrium polium* phytochemicals

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**Article history:**
Received 4 September 2015
Received in revised form 1 November 2015
Accepted 9 November 2015
Available online 5 December 2015

**Keywords:**
*Teucrium polium*
Lamiaceae
Antioxidant activity
Mosher’s reaction
Sesquiterpenes
Anti-tumor activity

**Abstract**
Chemical characterization as well as antioxidant and anti-tumor activity are reported for isolated metabolites from *Teucrium polium* (Lamiaceae). Structures were identified using standard MS and NMR spectroscopic methods. Sesquiterpene absolute stereochemistry was determined based on a modified Mosher’s reaction. Biological activity was evaluated by a cupric reducing antioxidant capacity (CUPRAC) assay and select compounds screened for anti-tumor activity. (1R,45,10R) 10,11-dimethyl-dicyclohex-5-(6)-en-1,4-diol-7-one and (R)-mandelonitrile-β-laminaribioside, together with ten previously reported compounds were identified. Antioxidant versus tumor-inhibition relationships was examined.

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http://dx.doi.org/10.1016/j.phytol.2015.11.007
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1. Introduction

Oxidants are ubiquitous in biological systems and can cause significant damage ranging from reversible biochemical modifications to permanent DNA alterations. In fact, ailments such as cardiovascular diseases, cancer and aging can result from such oxidative damage (Rahal et al., 2013). While biological systems have antioxidants to minimize such cellular oxidative damage, including enzymes (e.g. catalase) and organic compounds (metallothionein and ascorbic acid) (Klimp et al., 2002), excessive generation of oxidants can overload protective mechanisms of an organism (Rahal et al., 2013). Exogenous antioxidants can assist in reducing such oxidative damage and indeed a plethora of antioxidants exist ranging from water-soluble flavonoid glycosides to water-insoluble alpha-tocopherol.

The array of structurally different exogenous antioxidants points to a complexity of structural motifs responsible for antioxidant activity. In fact, simple biochemical modifications such as aglycone methylation and/or glycosylation structure–activity relationships have not been fully resolved. For example, with polyphenol glycosides, the number of sugars (Fiol et al., 2012), and/or phenolic groups (Siquet et al., 2006) can have a bearing on antioxidant activity. It has been suggested that free phenolic hydroxyl groups form stabilized phenoxyl radical intermediates increasing activity (Christodouleas et al., 2009). Indeed 0-modifications of flavonoid hydroxyl substitutions can directly reduce flavonoid antioxidant activity (Cao et al., 1997). For phenylpropanoid glycosides the degree of glycosylation has been shown to influence antioxidant activity (Rahman et al., 2006). Antioxidant activity can be additionally affected by the cellular environment with some antioxidants functioning both as pro-oxidants and antioxidants. For example, in tumor cells vitamin C acts as pro-oxidants producing hydrogen peroxide that can toxify the cell; whereas, in healthy cells the metabolite functions as a protective antioxidant (Casciari et al., 2001). Additional empirical-based structure–reactivity relationships are necessary to predict antioxidant activity based on characteristic chemical motifs.

The medicinal plant Tecricum polium, rich in antioxidants, was selected as a promising source of polyphenolic glycosides to examine structure–reactivity relationships of aglycone and sugar substitutions with antioxidant activity. Indeed, several glycosides (e.g. phenylpropanoid and iridoid) and aglycones (e.g. sesquiterpenes) have already been isolated from T. polium (Cozzani et al., 2005; De Marino et al., 2012; Elmasri et al., 2015a; Elmasri et al., 2015b). Building on such antioxidant chemical studies, structure–activity relationships were examined for phenylpropanoid metabolites isolated from T. polium. The compounds isolated including newly identified sesquiterpene and mandelonitrile glycoside (Fig. 1) allowed for anti-oxidation activity to be examined for comparable structures in methylation and glycosylation substitutions. In addition to investigating structure–antioxidant relationships, metabolite cytotoxicity against tumor cell lines was examined to provide insight into possible links between antioxidant and anti-tumor activity. Compound cytotoxicity was evaluated using a human tumor screen including cell lines for leukemia, melanoma, ovarian, breast, colon, lung, CNS, renal and prostate cancers (Shoemaker, 2006).

2. Materials and methods

2.1. General procedures

Optical rotations were measured in MeOH on an Autopol IV automatic polarimeter (Rudolph Research Analytical) equipped with a 10 cm microcell and a sodium lamp (λ_max = 589 nm). UV data were obtained on a Genesy 20 spectrophotometer. IR (KBr) spectra were recorded on a ThermoNicolet model IR 100 spectrophotometer. NMR spectra were obtained on a Joel NMR spectrometer equipped with Delta software; chemical shifts were reported in δ (ppm) and J coupling in Hz. The 13C NMR multiplicities were determined by DEPT experiments. NOE measurements were obtained from 2D NOESY experiments. One-bond heteronuclear 1H–13C connectivities were determined by HMOC, and two- and three-bond 1H–13C connectivities were determined by HMQC experiment. HRESIMS was performed on a Dionex Ultimate 3000 UHPLC system interfaced with an LTQ Orbitrap Velos (Thermo Scientific, Pittsburgh, PA, USA) mass spectrometer. Data were processed using Xcalibur Qual browser software (Thermo Scientific, Pittsburgh, PA, USA). GCMS analysis was performed on an ISQ QD Single Quadrupole GC–MS system and data were processed using Xcalibur software (Thermo Scientific, Pittsburgh, PA, USA). HPLC was performed using a prep-C18 column (21.2 × 250 mm, 10 μm) on an Agilent 1100 apparatus equipped with a Rhodyne injector and with UV detectors. Column chromatography was carried out using EMD silica gel 60 (70–230 mesh). Analytical TLC was performed on EMD silica gel 60 F254 sheets, 0.25 mm thick.

2.2. Reagent and chemicals

Trolox (purity 97%), α-tocopherol (purity >97%), butylated hydroxyanisole (BHA) (purity >98.5%), neocuproine (purity 99%), and ammonium acetate (purity ≥97%) were purchased from Fisher Scientific Inc.

2.3. Plant material

Aerial parts of T. polium were collected in June 2010, from North Sinai, Egypt and authenticated by ME Hegazy (National Research Centre, Egypt). A voucher specimen has deposited in the herbarium of St. Katherine protectorate, Egypt (voucher ID: SK-105).

2.4. Extraction and isolation

Air-dried aerial plant tissue (2 kg) was crushed. The resulted powder was extracted at room temperature with CH2Cl2–MeOH (1:1). The solvent was evaporated and the residue (210 g) subjected to silica gel CC eluting with n-hexanes, CH2Cl2, and MeOH in increasing order of polarity up to 15% MeOH in CH2Cl2 to afford 398 fractions. Based on metabolite complexity of chromatographic analysis, fractions A (225–232), B (250–265), and C (284–293) were combined and further purified for spectroscopic analysis and biological testing.

Fraction A (13 g) was concentrated in vacuo and subjected to silica gel CC eluting with a CH2Cl2–MeOH gradient (75:25) up to 100% MeOH; fractions were monitored by TLC with CH2Cl2–MeOH–H2O (6:2:0.5) to afford 42 fractions. Fractions 25–40 (3.2 g) were pooled and purified by reversed phase (RP) HPLC eluting with MeOH–H2O (0.1% HCHO) (25:75) to afford 1 (4.8 mg), 2 (4.8 mg), 3 (14 mg), 10 (12.2 mg), 11 (3.5 mg), and 12 (21 mg).

Fraction B (9 g) was concentrated in vacuo and subjected to silica gel CC eluting with a gradient of CH2Cl2–MeOH starting with (9:1) up to (0.5:9.5); fractions were monitored by TLC with CH2Cl2–MeOH–H2O (3:5:0.5) to afford 73 fractions. Sub-fractions 18–30 (1.5 g) were pooled and purified by RP HPLC eluting with an isocratic MeOH–H2O system (35:65) to afford 5 (21 mg).

Fraction C (6 g) was concentrated in vacuo and subjected to silica gel CC eluting with a CH2Cl2–MeOH (7:2) gradient up to CH2Cl2–MeOH (1:9); fractions were monitored by TLC with CH2Cl2–MeOH–H2O (6:2:0.5) to afford 46 fractions. Fractions
3. Results and discussion

The CH$_2$Cl$_2$-MeOH extract of T. polium aerial plant material was partitioned with a gradient of n-hexane, dichloromethane, and methanol. Compounds from the eluted fractions were purified using a combination of Sephadex, silica gel CC and RP-HPLC. The absolute stereochirality was assigned for 1, 3 and 4 for the first time.

Compound 1 was isolated as a yellow amorphous powder, with a specific rotation of $[\alpha]_D^{25} = -7.20$ (c 0.25, MeOH). The HR-ESI-MS exhibited a molecular ion peak [M+H]$^+$ at m/z 211.1330 (calc. 211.1329), and [M+Na]$^+$ at m/z 233.1149 (calc. 233.1148) suggesting a molecular formula of C$_{13}$H$_{18}$O$_3$. $^1$H NMR and DEPT spectra exhibited 12 signals corresponding to two methyls, four methylenes, a carbonyl, sp$^2$ hybridized carbon, an oxygenated methane, and two quaternary carbons (one oxygenated and one aliphatic). The $^1$H NMR spectrum displayed signals corresponding to two tertiary methyl groups at $\delta$H 1.33, 1.38 (both s, 3H), an olefinic proton at $\delta$H 5.99 (s, 1H), and an oxygenated methane proton at $\delta$H 3.32 (dd, J = 4.12 Hz, 1H). An alpha–beta unsaturated carbonyl was identified based on characteristic NMR signals and assigned to C-5–C-7 (Table 1). HMBC correlations between H-6 ($\delta$H 5.99) and 34.4 allowed for the assignment of C-8 (Fig. 2); H-8 were identified by HMQC (Table 1). $^1$H–$^1$H COSY correlations between H-8 at 2.55 and Hb-8 at $\delta$H 2.31 correlated with $\delta$H 2.18 and 1.76 allowing for the assignment of H-9 (Fig. 2). This was supported by HMBC correlations between H-9 and C-8/C-7 and between H-28 and C-9/C-10 confirming the presence of an alpha-beta-unsaturated cyclohexanone substructure. HMBC correlations from H-8 to $\delta$C 42.6 and 37.5 and from H-9 to $\delta$C 42.6, 17.8, 79.1, and 171.7 allowed for assignment of C-10, C-11, and C-1. This was supported by HMBC from $\delta$C 32.3 (H-1) to C-5, C-9, C-10, and C-11. COSY correlation between H-1 and $\delta$H 1.59 (H-2) and in turn H-2 and $\delta$H 1.51 (H-3a) and 1.87 (H-3b) allowed for H-1–H-3 assignments. HMBC confirmed these assignments with H-1 correlating with $\delta$C 26.7 (C-2) and 38.3 (C-3). HMBC correlations were also observed between $\delta$H 3 and $\delta$C 71.2 and 28.5 allowed for assignment of C-4/C-12, respectively.

Relative stereochirality was resolved by NOESY with correlations between Me-12 and H-3a ($\alpha$), H-3a and H-1, and Me-11 and H-9a ($\alpha$) and H-2 (Fig. 3); this implied that OH-1 and Me-12 are on the same molecular face. A modified Mosher’s reaction (Ohtani et al., 1991) was performed to determine the absolute configuration of the secondary alcohol at C-1. Treatment of two aliquots of 2 with (S)- and (R)-MTPA chloride afforded the corresponding esters 1a and 1b, with molecular ion peaks at m/z 427.1702 and 427.1701, respectively, consistent with successful derivatization. The pattern of $\delta$C (S–R) values (Fig. 4) allowed for determining the absolute configuration at C-1 to be $R$ and the stereochemistry based on NMR and Mosher data was assigned to 1R, 4S, and 10R as shown in Fig. 1. The structure of 1 is 1R, 4S, 10R 10.11-dimethyl-dicyclohex-5-(6)-en-1,4-diol–7-one, a new natural product.

Compound 2 was isolated as a yellow amorphous powder with a specific rotation of $[\alpha]_D^{25} = -13.84$ (c 0.13, MeOH). HR-ESI–MS exhibited a molecular ion peak [M+Na]$^+$ at m/z 480.1486 (calc. 480.1481), suggesting a molecular formula C$_{27}$H$_{30}$NO$_4$. The $^1$H NMR spectrum displayed an AA’BB’ ring system assigned to a mono-substituted aromatic ring at $\delta$H 7.59 (m, J = 18.3 Hz, 1H) and 7.43 (m, J = 2.29, 1.83 Hz, 4H). In addition, a downfield proton at $\delta$H 6.02 (s, 1H), was consistent with a nitrogenous substitution. Two anomeric signals at $\delta$H 4.57 (d, J = 7.79 Hz, 1H), 4.79 (d, J = 7.33 Hz, 1H) were consistent with a $\beta$-glucose sequence as observed after acid hydrolysis via GC comparison with an authentic standard. HMBC correlations from H-1’ to C-7 at $\delta$C 68.1 and from H-1” to C-3’ supported the linkage of both glucose units to C-7 and C-3,’

2.5. Acid hydrolysis

A solution of 2 (1 mg in H$_2$O: dioxane (1:1) with 1 N HCl (1 mL total volume)) was heated at 80 °C for 2 h. After cooling, the reaction mixture was neutralized using Amberlite IRA-68, the resin removed by filtration and the filtrate extracted with EtOAc. The aqueous layer was concentrated and the sugars were identified by TLC with n-hexane:EtOAc:MeOH:AcOH:H$_2$O (1:4:2.5:0.5) by comparison with authentic standards (Wang et al., 2004).

2.6. Gas chromatography analysis

For sugar identification, an aqueous aliquot was silylated with N-trimethylsilylimidazole (TMSI) for 2 h at room temperature. After reaction termination with dist. H$_2$O, the mixture was partitioned with n-hexane and the organic layer was analyzed by GCMS. For sugar configuration identification, L-cysteine methyl ester hydrochloride in pyridine (0.2 mL, 0.06 M) was added to the aqueous layer, stirred at 60 °C for 1 h and incubated at RT for 2 h with TMSI (0.2 mL). The reaction mixture was partitioned with n-hexane and distilled H$_2$O, and the organic layer was analyzed by GCMS (Elmasri et al., 2015a). D-Glucose was confirmed by retention-time comparison (26.2 min) with derivatized authentic standards.

2.7. Preparation of (R) and (S)-MTPA esters

An aliquot of 1, 3 and 4 (2 mg each) were dissolved in CHCl$_3$ (2 mL) with dry pyridine under N$_2$. (S)- or (R)-$\alpha$-methoxy-$\alpha$-(trifluoromethyl)phenylacetyl (MTPA) chloride (0.1 mL) was added to each aliquot. The reaction was stirred overnight, quenched with a saturated NaHCO$_3$ solution and the CHCl$_3$ layer was aqueous rinsed and dried under reduced pressure to afford the (R) and (S)-MTPA esters (Elmasri et al., 2015a).

2.8. Cu$^{2+}$ reduction assay

The cupric ion (Cu$^{2+}$) reducing method was used with slight modifications (Ak and Gulcin, 2008). To an aqueous CuCl$_2$ solution (0.01 M, 0.25 mL), ethanolic neocuprine (7.5 × 10$^{-3}$ M, 0.25 mL) and CH$_3$COONa (1 M, 0.25 mL, pH 6.5) along with different concentrations of the test compound (15–60 μM) or a synthetic positive control were combined. Absorbance was recorded at 450 nm, after 30 min. TEAC coefficients were calculated by dividing the extinction coefficient of the test compound (e) by that of trolox (Gungor et al., 2011; Nechifor et al., 2012).

2.9. Cytotoxicity screening

Anti-tumor screening was performed on 4, 5, 6, 10, and 12 at a single concentration (10 μM) at the National Cancer Institute according to standard procedures (Alley et al., 1988; Boyd and Paull, 1995; Grever et al., 1992; Shoemaker, 2006) with a NCI-60 DTP human tumor cell line screen (http://dtp.nci.nih.gov/branches/btb/wclsp.html).

2.10. Statistical analysis

Data were expressed as the mean ± standard deviation (SD) of three measurements. A Student t test was used to determine significant differences between means with a significant difference defined at $p \leq 0.05$. 

3-4 (250 mg) were pooled and purified by reversed phase (RP) HPLC eluting with gradient MeOH–H$_2$O system (41:59) to (50:50) to afford 6 (7.5 mg), 7 (2.8 mg), 8 (1.5 mg), and 9 (6.5 mg).
respectively (Fig. 2). These data indicate a mandelonitrile disaccharide similar to what has been previously reported (Neilson et al., 2011) except for the configuration at C-7. Prediction of the stereochemistry of cyanogens aglycone moiety was suggested by Nahrstedt based on chemical shift data (Huebel et al., 1981) and complexity of the anomeric proton signals (Seigler et al., 2002). This signal complexity is attributable to anisotropism of the neighboring aromatic ring. The δ1 of the (S)-configuration appears up-field relative to the (R)-configuration (Huebel et al., 1981). Examination of both 1H and 13C NMR spectral data and comparison with published mandelonitrile glycosides (Neilson et al., 2011) as well as 6 indicated an (R)-configuration at C-6. This was confirmed by an absence of higher-order spin multipletics which gives rise to an obscured doublet (observed as a multiplet) in the case of (S)-configurations (Huebel et al., 1981). On the basis of this spectral data, the structure was identified as (R)-mandelonitrile-β-laminarinbioside, a new natural product.

Compound 3 was isolated as a yellow amorphous powder, with a specific rotation of [α]D25 = +6.66 (c 0.45, MeOH). The HR-ESI–MS exhibited a molecular ion peak [M+Na]+ at m/z 289.1406 (calc. 289.1410) suggesting a molecular formula of C15H22O6. Based on 1H and 13C (Table 1), 2D NMR data (Fig. 2), similarity to a previously reported germacranolide is established (Gordon et al., 1981). Because the absolute stereochemistry is inexorably linked to biological activity and the enantiomers of a given chiral molecules should be considered two different drugs (McConathy and Owens, 2003), a modified Mosher’s reaction (Ohtani et al., 1991) was performed to establish the absolute configuration of 3. Treatment of two aliquots of 1 with (S)- and (R)-MTPA chloride afforded the corresponding diesters 3a and 3b, with molecular ion peaks at m/z 721.2191 and 721.2196 (calc. 721.2206), respectively, consistent with derivatized products. The pattern of δ1 (S–R) values (Fig. 4) allowed for the determination of R at C-1 and C-6. Therefore 3 was assigned as (1R,6R,7R,8S,11R)-1,6-dihydroxy-4,11-dimethyl-germacran-4(5), 10(14)-di-en-8,12-olide.

The relative stereochemistry of 4 was previously reported (Sanz and Marco, 1991) while the absolute stereochemistry was established here again based on a modified Mosher’s method. Treatment of two aliquots of 4 with (S)- and (R)-MTPA chloride afforded the corresponding esters 4a and 4b, respectively. The pattern of δ1 (S–R) values (Fig. 4) allowed for determining the absolute configuration at C-6 to be R and other position were assigned to 1R, 4S, 5S, 6R, 7S, and 10R as shown in Fig. 1. Thus, the structure of 4 was established as (10R,1R,4S,5S,6R,7S)-4,10-diepoxygermacran-6-ol.

In addition, eight previously reported compounds: 8-acetyl-harpagide (5) (Takeda et al., 1987) polumoside (6) (Elmasri et al., 2014), 2-(3-hydroxy-4-methoxyphenyl)-ethyl-O-(αL-rhamnosyl)-

Table 1

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* Signal patterns are unclear due to overlapping.

Fig. 1. Chemically identified metabolites. Consensus structures of newly identified phytochemicals.

Fig. 2. HMBC and COSY observed with compounds 1–2. HMBC (solid arrow) and COSY (solid, black line) correlations observed with compounds 1–2.

Fig. 3. NOESY correlations observed with compounds 1.

All isolated compounds were assayed for anti-oxidant activity based on a well-established CUPRAC assay with 2 exhibiting low activity with a TEAC value of 0.11 ± 0.03 μmol TE/100 μmol compound in comparison with the positive control trolox. Since a series of the isolated phenylpropanoid metabolites varied in their glycosylation and methylation functionality, structure–antioxidant relationships for compounds 6–9 were examined. Increasing sugar units with accompanying free-phenolic-hydroxyl groups increased anti-oxidant activity. Compound 6 exhibited higher antioxidant capacity than any of the other tri-saccharide phenylpropanoids assayed. Specifically activities descended in order from 6 (three sugars, no methylation) > 7 (three sugars, single methylation) > 8 (three sugars, double methylation) and > 9 (two sugars, no methylation). Consistent with this trend, polumoside B (De Marinho et al., 2012) (4 sugars, no methylation) has an antioxidant capacity greater than 6 suggesting that increasing the number of sugar moieties enhances antioxidant activity. Interestingly, NaOH disruption of polysaccharide spatial structure as observed by atomic force microscopy has been shown to be correlated with reduced antioxidant activity (Liu et al., 2011). Moreover, 6 and 9 with free ortho-dihydroxyl groups showed higher activity than the positive controls, trolox and α-tocopherol with non-adjacent hydroxyl groups (Fig. 5). These results are consistent with previous reports that increasing free phenolic hydroxyl pairs enhances antioxidant activity (Siquet et al., 2006; Krishnamachari et al., 2004). Such phenolic hydroxyl pairs are thought to form hydrogen bond between adjacent groups that can stabilize phenox radical intermediates (Christodoulas et al., 2009). Hydroxyl methylation decreases antioxidant activity as it destabilizes the intermediate by disrupting hydrogen bonding.

Compounds 4–6, 10 and 12 were also assayed for tumor cytotoxicity; growth inhibition percent (GIP) was compared to nontoxic drug control and relative to time zero number of cells (Table 2). This assay allows for detection of both growth inhibition percent (GIP) (values between 0 and 100) and lethality (values less than 0). Polumoside (6) showed GIP of −10, −9, −10, −12, −12 against NCI-H322M, SF-539, OVCA-4, OVCA-8, and ACHN cell lines, respectively. On the other hand, 6 showed a GIP of 12, 10, and 10 against NCI-H460, SNB-19, and SK-OV-3 cell lines, respectively. Teucardoside (12) showed GIP of −12, −9, −10, −16 and −25 against NCI/ADR-RES, NCI-H322M, SK-MEL-28, HOP-92 and RXF 393, respectively. However, 12 also exhibited a GIP of 10, 11, 13, 14, 12, 19, and 10 against SK, SK-OV-3, NCI-H23, NCI-H460, SNB-19, and SK-OV-3.

LOX IMVI, and UAACC-257, respectively. Compound 5 has induced the growth of cancer cell lines: HOP-92 and HCC-2998 by 29 and 16%, respectively, and decreased the growth of SNB-75 cell line by 17%. Notably, poliumoside and teucardoside, which have significant antioxidant properties, resulted in varied effects on several cancer cell lines. This inconsistent connection between anti-tumor and antioxidant activity observed with the compounds isolated here from T. polium is consistent with in vitro studies (Patterson et al., 1997). In one case, antioxidant supplements had decreased the risk of death from gastric cancer but not from esophageal cancer. However, risk of developing gastric cancer and/or esophageal cancer were not affected by antioxidant supplementation (Blot et al., 1993). In another study, an increase in the incidence of lung cancer occurred with beta-carotene supplements; in contrast, alpha-tocopherol had no effect on lung cancer incidence (Heinonen et al., 1994). Antioxidants supplements in combination with cancer therapy can alter the effectiveness or reduces the toxicity of specific drugs (Lawenda et al., 2008). Clearly, additional studies are needed to clarify possible connections between antioxidant supplements and tumor progression.

3.1. (1R,4S,10R) 10,11-dimethyl-dicyclohex-5-(6-en)-1,4-diol-7-one (1)

Yellow amorphous powder. [α]D 25 = 7.20 (c 0.25, MeOH). UVmax 286. IR (KBr) cm−1: 3405, 2933, 1724, 1685, 1594, 1374, 1240, 1042,
3.3. (S)-MTPA ester of 1 (1b)

1H NMR (CD3OD, 400 MHz): 4.80 (1H, dd, H-1), 2.25 (2H, m*, H-2), 1.88 (2H, m*, H-2), 1.69 (2H, m, H-3a), 6.02 (1H, s, H-6), 2.39 (1H, m, H-8a), 2.32 (1H, m, H-8b), 1.75 (1H, m, H-9a), 1.38 (3H, brs, H-11), 1.37 (3H, s, H-12); an asterisks (*) indicates overlapping signals. HRESIMS m/z: [M+H]+ 427.1701 (calc. 427.1727).

3.4. (R)-mandelonitrile-β-laminaribioside (2)

Yellow amorphous powder. [α]D<sup>25</sup> = −13.84 (c 0.13, MeOH). UV<sub>max</sub> 283. IR (KBr) cm<sup>−1</sup>: 3288, 2932, 1713, 1598, 1077, 609. HRESIMS m/z: [M+Na]<sup>+</sup> 480.1486 (calc.480.1481) for C<sub>20</sub>H<sub>27</sub>NO<sub>11</sub>. Elemental analysis C, 52.51; H, 5.95; N, 3.06; O, 38.47.

3.5. (R)-MTPA ester of 3 (3a)

1H NMR (CDCl<sub>3</sub>, 400 MHz): 4.83(1H, d, H-1), 1.96 (2H, m*, H-2), 2.18 (2H, m, H-3), 5.10 (1H, m, H-5), 5.34 (1H, d, H-6), 2.25 (1H, m*, H-7), 3.84 (1H, m, H-8), 3.12 (1H, m, H-9a), 5.37 (1H, brs, H-14a), 5.27 (1H, brd, H-14b), 1.72 (3H, s, H-15); an asterisks (*) indicates overlapping signals. HRESIMS m/z: [M+Na]<sup>+</sup> 721.2189 (calc. 721.2206).

3.6. (S)-MTPA ester of 3 (3b)

1H NMR (CDCl3, 400 MHz): 4.80 (1H, d, H-1), 2.08 (2H, m*, H-2), 2.22 (2H, m, H-3), 5.20 (1H, m, H-5), 5.36 (1H, d, H-6), 2.18 (1H, m*, H-7), 3.79 (1H, m, H-8), 3.11 (1H, m, H-9a), 5.18 (1H, brs, H-14a), 5.17 (1H, brd, H-14b), 1.72 (3H, s, H-15); an asterisks (*) indicates overlapping signals. HRESIMS m/z: [M+Na]<sup>+</sup> 721.2191 (calc. 721.2206).

3.7. (R)-MTPA ester of 4 (4a)

1H NMR (CDCl3, 400 MHz): 2.98 (1H, m, H-1), 2.28 (1H, m*, H-2), 1.14 (1H, m, H-3a), 2.09 (1H, m, H-3b), 2.94 (1H, d, H-5), 5.22 (1H, dd, H-6), 1.06 (1H, m, H-7), 1.81 (1H, m*, H-8a), 2.20 (1H, m, H-9a), 0.85 (3H, d, H-12), 0.92 (3H, d, H-13), 1.45 (1H, s, H-14), 1.29 (3H, s, H-15); an asterisks (*) indicates overlapping signals.

3.8. (S)-MTPA ester of 4 (4b)

1H NMR (CDCl3, 400 MHz): 2.98 (1H, m, H-1), 2.30 (1H, m*, H-2), 1.18 (1H, m, H-3a), 2.12 (1H, m, H-3b), 2.85 (1H, d, H-5), 5.24 (1H, dd, H-6), 1.02 (1H, m, H-7), 1.75 (1H, m*, H-8a), 2.19 (1H, m, H-9a), 0.92 (3H, d, H-12), 0.95 (3H, d, H-13), 1.46 (1H, s, H-14), 1.25 (3H, s, H-15); an asterisks (*) indicates overlapping signals.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The authors are grateful for in vitro screening by the National Cancer Institute (Bethesda, MD) [http://dtp.cancer.gov]. Research was supported in part by the Robert Welch Foundation (D-1478), NSF equipment grant CHE-1048553 and NSF CRIF program.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytol.2015.11.007.
References